Total synthesis and structural revision of aeruginosin KT608A

Scherer, Manuel ; Gademann, Karl

Abstract: The synthesis of the presumed structure of aeruginosin KT608A was accomplished for the first time. The unusual D-diepi-Choi core was prepared from tyrosine via C-H activation and heterogeneous hydrogenation. Due to differences in the spectral data of synthetic and natural samples, a revised structure featuring L-diepi-Choi was proposed, which was synthesized and confirmed to be identical. Based on these findings, revised structures for six additional aeruginosins (KT608B, KT650, GH553, DA495A, DA511 and KB676) are presented.

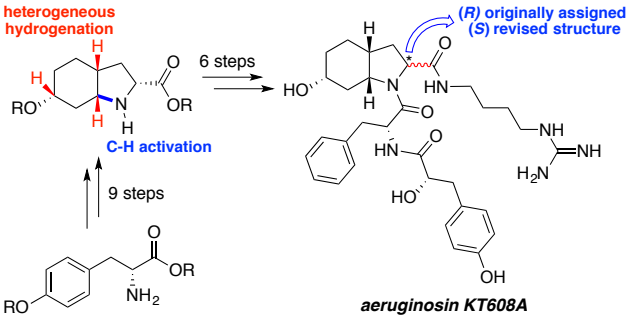
DOI: https://doi.org/10.1021/acs.orglett.7b01822

Total Synthesis and Structural Revision of Aeruginosin KT608A

Manuel Scherer[†,§] and Karl Gademann*[,†]

[†]Department of Chemistry, University of Zurich, 8057 Zurich, Switzerland

Supporting Information Placeholder



ABSTRACT: The synthesis of the presumed structure of aeruginosin KT608A was accomplished for the first time. The unusual D-*diepi*-Choi core was prepared from tyrosine *via* C-H activation and heterogeneous hydrogenation. Due to differences in the spectral data of synthetic and natural samples, a revised structure featuring L-*diepi*-Choi was proposed, which was synthesized and confirmed to be identical. Based on these findings, revised structures for six additional aeruginosins (KT608B, KT650, GH553, DA495A, DA511 and KB676) are presented.

Aeruginosins constitute a group of linear modified tetrapeptides isolated form cyanobacteria and marine sponges. These compounds are characterized by a central 6-hydroxyoctahydroindole-2-carboxylic acid (Choi) motif which is decorated with an arginine mimicking residue on the C-terminus. The N-terminus often features a hydrophobic amino acid on the second position and a phenyllactic acid derivative or a fatty acid on the first position. Since the first isolation of an aeruginosin in 1994 by Murakami and coworkers[1] this class of natural products gained attention as protease inhibitors,[2] and more recently as potent biotoxins.[3] Today, around 60 different aeruginosins are known, with many of them possessing modifications such as additional halogen atoms, sulfate, and/or glycosyl groups.[2] The vast majority (>50) of aeruginosins consist of a Choi moiety with (2*S*,3a*S*,6*R*,7a*S*) configuration, referred to as L-Choi (Figure 1). Recently, structures with variations of the Choi residue have been assigned to various congeners. Aeruginosin EI461 displays the inverted (*R*) configuration both at the 3a and 7a positions, compared to the (*S*) configuration in L-Choi.[4] Consequently, this Choi isomer is referred to as L-*diepi*-Choi. Aeruginosins DA495A, DA511 and KB676 have been assigned an inverted configuration at the 6-position, thus resulting in L-6-*epi*-Choi (Figure 1).[5]

Aeruginosin KT608A (**1**) was isolated along with aeruginosins KT608B, KT650 and GH553 from *Microcystis aeruginosa* bloom material from Lake Kinneret, Israel.[6] These aeruginosins have been assigned a D configuration of the Choi motif (inverted at the 2 position) which is unique compared to other aeruginosins. Besides the D configuration, the Choi unit of KT608A (**1**) was suggested to differ additionally from the common L-Choi by epimerization at the 3a and 7a positions (D-*diepi*-Choi). Further structural features of aeruginosin KT608A (**1**) are an agmatine (Agma) residue on the C-terminus and a D-phenylalanine and hydroxyphenyllactic acid moiety on the N-terminus (Figure 2).[6] In the past, large efforts have been made aiming at the synthetic preparation of L-Choi containing aeruginosins,[7] whereas the synthesis of D-*diepi*-Choi congeners has rarely been explored. In addition, to the best of our knowledge, the presence of the D-*diepi*-Choi in aeruginosins has not yet been confirmed by total synthesis.

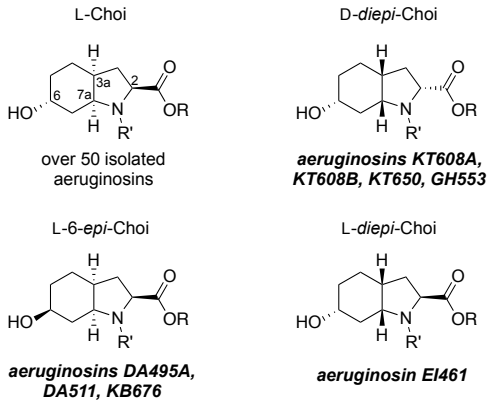


Figure 1. Structures of the Choi nucleus in different aeruginosins.

In this study, we report the first total synthesis of the putative structure of aeruginosin KT608A (**1**), and after disclosing evidence for the incorrect assignment of the configuration, an alternative structure (**2**) was proposed and verified by total synthesis (Figure 2). In addition, we disclose a new strategy towards the Choi unit based on C-H activation and stereoselective hydrogenation reactions.

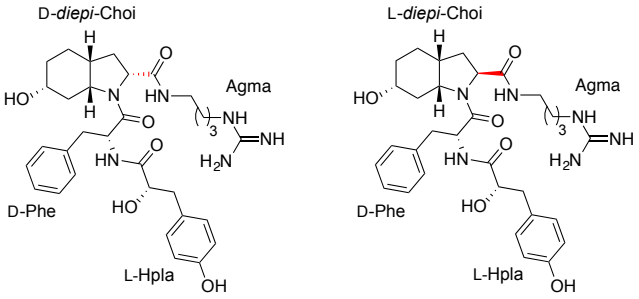
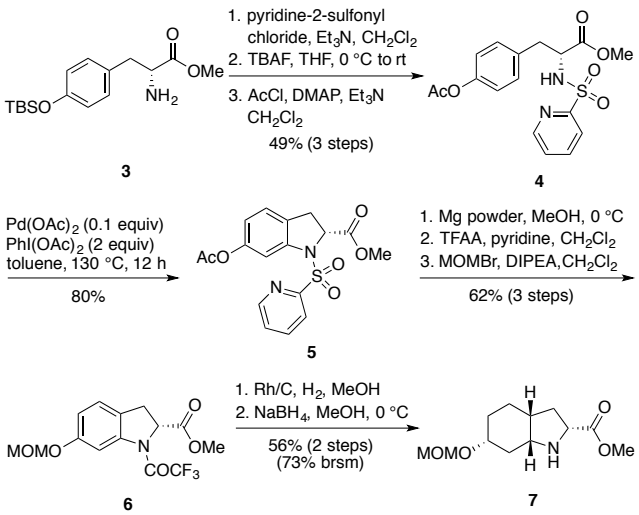


Figure 2. Originally assigned (**1**) and revised (**2**) structures of aeruginosin KT608A.

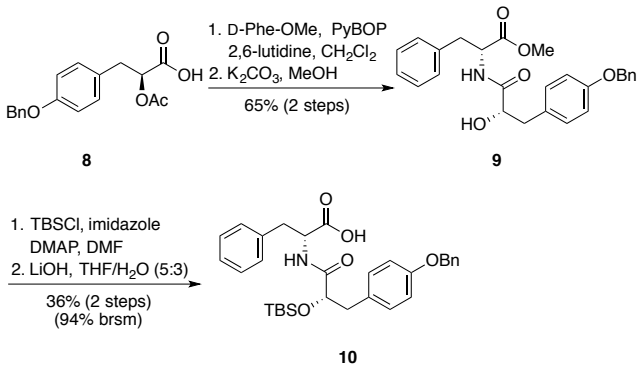
The synthesis of aeruginosin KT608A (**1**) commenced with the attachment of pyridine-2-sulfonyl chloride[8] to TBS-protected tyrosine methyl ester **3**[9] (Scheme 1). 2-Pyridine-sulfonamides have been successfully introduced as directing group for intramolecular aminations of arylethylamines by Yu and co-workers.[10] Since the intramolecular amination of **3** to the corresponding indoline only proceeded in moderate yields around 50%, the TBS protecting group was replaced by the more electron-withdrawing acetyl group. To this goal, derivative **3** was treated with TBAF solution followed by acetylation of the obtained alcohol using AcCl and DMAP to give acetyl ester **4**. Pleasingly, the intramolecular amination of **4** could be achieved at elevated temperature with $Pd(OAc)_2$ as catalyst and $PhI(OAc)_2$ as oxidant to give indoline **5** in a very good yield of 80%. Treatment of indoline **5** with methanolic Mg^0 solution led to the cleavage of the sulfonamide directing group and acetyl ester. All attempts to perform the reduction of the indoline to the octahydroindole at this stage were not rewarded with any success. The low reactivity of the indoline towards hydrogenation was assigned to the presence of the free amino- and hydroxy groups. Thus, the amine was trifluoroacetylated and the hydroxyl group equipped with a MOM protecting group to give fully protected indoline **6**. Fortunately, indoline **6** could be readily transformed to the octahydroindole by heterogeneous hydrogenation using Rh/C as catalyst at 15 bar H_2 pressure.[11] Thereby, the carboxyl group of **6** led to a substrate controlled all-*syn* addition of the introduced hydrogen atoms and thus to the formation of only one diastereoisomer. Due to the modest stability of the trifluoroacetyl amide under the applied hydrogenation conditions, the reaction was stopped before reaching full conversion to enhance yields. Subsequent removal of the trifluoroacetyl group using $NaBH_4$ furnished D-*diepi*-Choi derivative **7** (Scheme 1).

Scheme 1. Synthesis of D-*diepi*-Choi Building Block 7



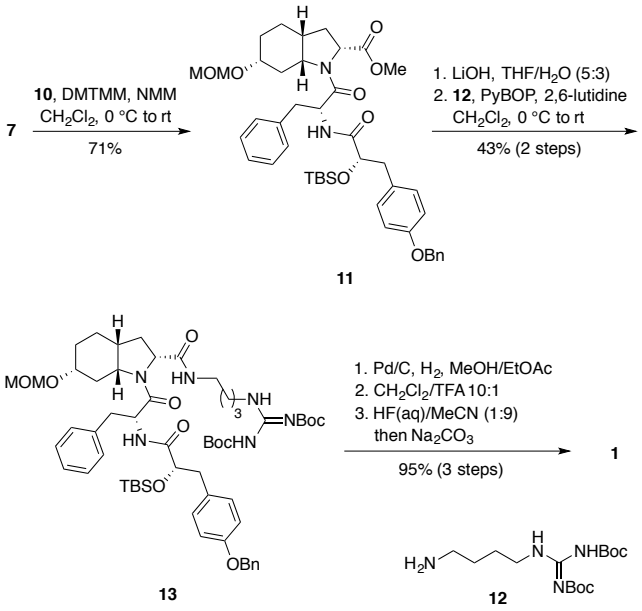
For the introduction of the phenyllactic acid (Hpla) and the phenylalanine (Phe) residues on the N-terminus of aeruginosin KT608A (**1**) we envisioned the incorporation of these two moieties as one building block. The required Hpla-Phe-OH fragment **10** was prepared over four synthetic steps from known phenyllactic acid derivative **8**[12] (Scheme 2). Peptide coupling of acid **8** with D-phenylalanine methyl ester followed by selective saponification of the acetyl ester afforded Hpla-Phe-OMe dipeptide **9** in 65% yield over two steps. Subsequent TBS protection of the obtained alcohol followed by hydrolysis of the methyl ester yielded Hpla-Phe-OH building block **10**.

Scheme 2. Synthesis of Hpla-Phe-OH Fragment 10



The assembly of the different building blocks was initiated by coupling D-*diepi*-Choi derivative **7** with Hpla-Phe-OH fragment **10** (Scheme 3). For the peptide bond formation DMTMM was used as coupling reagent to give tripeptide **11** in good yield with no observable isomerization.[13] Hydrolysis of the methyl ester and subsequent coupling of the acid with agmatine side chain **12**[14] provided tetrapeptide **13** in moderate yield. Global deprotection commenced with catalytic hydrogenation to remove the Bn group. Subsequent removal of the Boc and MOM groups was achieved by treatment with diluted TFA solution. Surprisingly, the TBS group proved to be stable under the applied acidic conditions. Thus, additional treatment with diluted HF solution was required to complete the cleavage of the TBS group. To hydrolyze partially formed trifluoroacetyl ester, formed during the second deprotection step, the reaction mixture was treated with aqueous Na_2CO_3 solution to give pure aeruginosin KT608A (**1**) in excellent yield.

Scheme 3. Synthesis of the Presumed Structure of Aeruginosin KT608A (1)



However, comparing the NMR spectral data of the synthesized sample to the values reported for the isolated aeruginosin KT608A revealed large deviations. The most apparent difference in the NMR spectra arises from the ratio of the *cis* and *trans* rotamer characteristics for aeruginosins. The NMR spectra of isolated aeruginosin KT608A appear in a 1:1 ratio at 300K in DMSO-d_6, whereas spectra of the synthesized sample revealed a ratio of 3:1 favoring the *trans* rotamer. In addition, the observed chemical shifts were different from the reported values as well: while Choi H-2, H-3α, H-3β, H-7(eq) and H-7(ax) resonance at 4.24, 1.81, 1.96, 1.60, and 1.90 ppm in the synthetic sample of aeruginosin, the signals of their equivalents in the isolated sample have been reported to appear at 4.82, 2.38, 1.70, 2.37, and 0.83 ppm.[15] Reviewing the literature data for several compounds revealed distinct similarities of the shifts belonging to the Choi unit of natural aeruginosin KT608A and the L-*diepi*-Choi signals of aeruginosin EI461.[4]

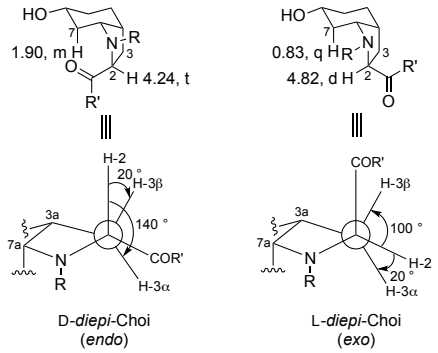
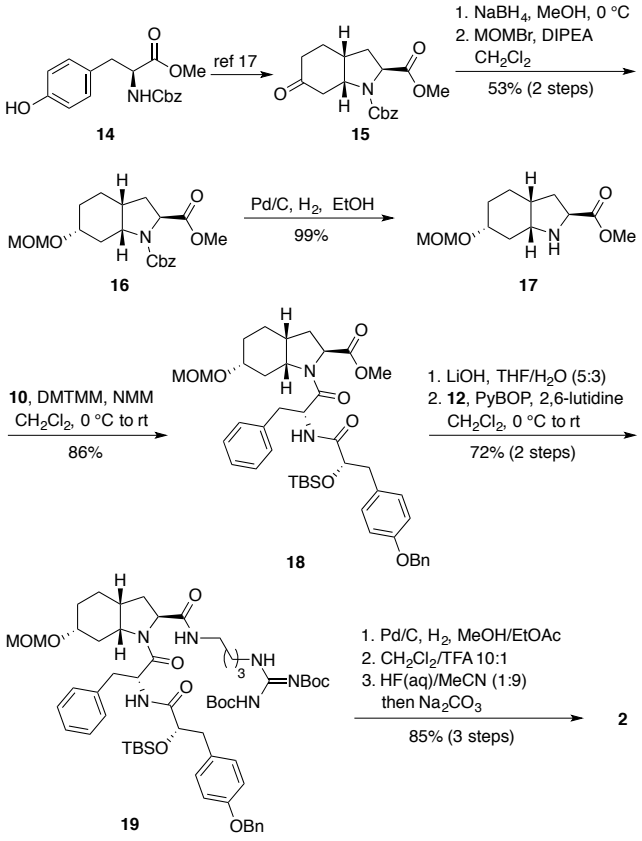


Figure 3. Differences in the chemical shifts of the Choi H-2 and H-7 in the D-*diepi* and L-*diepi*-Choi.

Due to the *endo* configuration of the D-*diepi*-Choi, the carbonyl group is in close proximity to H-7(ax) leading to a drastic deshielding thereof (m, 1.90 ppm). In the *exo* configuration of the L-*diepi*-Choi such an effect is missing, resulting in an upfield shifted signal of H-7(ax) (q, 0.83 ppm). In addition, the H-2 signal is strongly influenced by the configuration at C-2. In D-*diepi*-Choi, the dihedral angles of H2-C2-C3-H3α and H2-C2-C3-H3β correspond to 20 and 140°, respectively, resulting in a pseudo-triplet multiplicity of the H-2 signal in the ^{1}H NMR spectrum (t, 4.24 ppm). On the other hand, the equivalent dihedral angles in the L-*diepi*-Choi amount to 20 and 100°, respectively. As a result, the Choi H-2 signal in the L-*diepi*-Choi appears as a doublet (Figure 3).[4,16] To support the hypothesis that the structure of aeruginosin KT608A contains an L-*diepi*-Choi motif, the revised structure **2** of aeruginosin KT608A was prepared. To this goal, L-*diepi*-Choi derivative **17** needed to be accessed. Following procedures of Wipf and coworkers,[17] ketone **15** was prepared over five steps from tyrosine derivative **14**. Reduction of ketone **15** with $NaBH_4$ followed by protection of the resulting alcohol with a MOM group afforded octahydroindole **16**. Removal of the Cbz group gave L-*diepi*-Choi derivative **17** (Scheme 4). The assembly of the building blocks was performed analogously to the synthesis of **1**. Coupling of L-*diepi*-Choi derivative **17** to Hpla-Phe-OH fragment **10** provided tripeptide **18**. Hydrolysis of the L-*diepi*-Choi methyl ester followed by linkage of the acid to agmatine side chain **12** yielded tetrapeptide **19**. Global deprotection was carried out as mentioned above and afforded aeruginosin **2** consisting of a L-*diepi*-Choi core. The NMR spectral data of the revised structure **2** were in full agreement with values reported for the natural aeruginosin KT608A. This evidence strongly corroborates the hypothesis that aeruginosin KT608A contains a Choi motif with an L-*diepi* rather than a D-*diepi* configuration.

Scheme 4. Synthesis of the Revised Structure of Aeruginosin KT608A (2)



After revealing the revised structure of aeruginosin KT608A by total synthesis, we reviewed the NMR spectral data of aeruginosins KT608B, KT650, GH553, DA495A, DA511 and KB676 as published in the literature.[5,6] The NMR spectra of all these aeruginosins displayed characteristic signals assigned to the L-*diepi*-Choi rather than the D-*diepi*-Choi (for KT608B, KT650 and GH553) or the L-6-*epi*-Choi (for DA495A, DA511 and KB676), respectively. This strongly supports the hypothesis that the structures of these aeruginosins have also been assigned incorrectly and that an L-*diepi*-Choi configuration is present in all of these secondary metabolites.[18] Consequently, the existence of the D-*diepi*-Choi and the L-6-*epi*-Choi configuration in natural aeruginosins has to be questioned at present, based on the synthetic work and structural analysis reported in this study.

In conclusion, the total synthesis of the proposed structure **1** of aeruginosin KT608A is presented. The unique D-*diepi*-Choi core was prepared via C-H activation followed by heterogeneous hydrogenation. Differences in the NMR spectral data of synthesized and isolated aeruginosin KT608A led to a structural revision of the structure. The proposed revised structure **2** featuring an L-*diepi*-Choi core configuration was established by total synthesis. Furthermore, based on these synthetic studies combined with structural analysis by spectroscopic means, revised structures for six additional aeruginosins are proposed.

ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures, full characterization, and copies of all spectra. This material is free of charge via Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

* Email: karl.gademann@uzh.ch

Present Address

[§]Department of Chemistry, University of British Columbia, BC V6T 1Z1, Canada.

ACKNOWLEDGMENTS

We thank Simon Jurt (University of Zurich) for support with NMR spectroscopy measurements. Cedric Lüthi, Angela Amsler and Agron Ilazi (all University of Zurich) are gratefully acknowledged for skillful technical assistance.

REFERENCES

(1) Murakami, M.; Okita, Y.; Matsuda, H.; Okino, T.; Yamaguchi, K. *Tetrahedron Lett.* **1994**, *35*, 3129–3132.

(2) For a review on aeruginosins and their biological activity, see: Ersmark, K.; Del Valle, J. R.; Hanessian, S. *Angew. Chem. Int. Ed.* **2008**, *47*, 1202–1223.

(3) (a) Kohler, E.; Grundler, V.; Häussinger, D.; Kurmayer, R.; Gademann, K.; Pernthaler, J.; Blom, J. F. *Harmful Algae* **2014**, *39*, 154–160; (b) Scherer, M.; Bezold, D.; Gademann, K. *Angew. Chem. Int. Ed.* **2016**, *55*, 9427–9431.

(4) (a) Ploutno, A.; Shoshan, M.; Carmeli, S. *J. Nat. Prod.* **2002**, *65*, 973–978; (b) Valls, N.; Vallribera, M.; Carmeli, S.; Bonjoch, J. *Org. Lett.* **2003**, *5*, 447–450.

(5) (a) Adiv, S.; Carmeli, S. *J. Nat. Prod.* **2013**, *76*, 2307–2315; (b) Elkobi-Peer, S.; Carmeli, S. *Mar. Drugs* **2015**, *13*, 2347–2375.

(6) Lifshits, M.; Carmeli, S. *J. Nat. Prod.* **2012**, *75*, 209–219.

(7) For syntheses, see: (a) Valls, N.; Vallribera, M.; Font-Bardía, M.; Solans, X.; Bonjoch, J. *Tetrahedron Asymmetry* **2003**, *14*, 1241–1244; (b) Valls, N.; Lopez-Canet, M.; Vallribera, M.; Bonjoch, J. *J. Am. Chem. Soc.* **2000**, *122*, 11248–11249; (c) Hanessian, S.; Tremblay, M.; Petersen, J. F. W. *J. Am. Chem. Soc.* **2004**, *126*, 6064–6071; (d) Hanessian, S.; Del Valle, J. R.; Xue, Y. F.; Blomberg, N. *J. Am. Chem. Soc.* **2006**, *128*, 10491–10495; (e) Hanessian, S.; Margarita, R.; Hall, A.; Johnstone, S.; Tremblay, M.; Parlanti, L. *J. Am. Chem. Soc.* **2002**, *124*, 13342–13343; (f) Diethelm, S.; Schindler, C. S.; Carreira, E. M. *Org. Lett.* **2010**, *12*, 3950–3953; (g) Diethelm, S.; Schindler, C. S.; Carreira, E. M. *Chem. Eur. J.* **2014**, *20*, 6071–6080; (h) Trost, B. M.; Kaneko, T.; Andersen, N. G.; Tappertzhofen, C.; Fahr, B. *J. Am. Chem. Soc.* **2012**, *134*, 18944–18947; (i) Dailler, D.; Danoun, G.; Ourri, B.; Baudoin, O. *Chem. Eur. J.* **2015**, *21*, 9370–9378; (j) Dailler, D.; Danoun, G.; Baudoin, O. *Angew. Chem. Int. Ed.* **2015**, *54*, 4919–4922.

(8) Yang, D.; Mao, S.; Gao, Y.-R.; Guo, D.-D.; Guo, S.-H.; Li, B.; Wang, Y.-Q. *RSC Adv.* **2015**, *5*, 23727–23736.

(9) Cao, B.; Xiao, D.; Joullie, M. M. *Org. Lett.* **1999**, *1*, 1799–1801.

(10) Mei, T. S.; Leow, D.; Xiao, H.; Laforteza, B. N.; Yu, J. Q. *Org. Lett.* **2013**, *15*, 3058–3061.

(11) T. Mezei, M. Porcs-Makkay, G. Simig, *US Patent* **2004**, 198988 A1.

(12) Valls, N.; Vallribera, M.; López-Canet, M.; Bonjoch, J. *J. Org. Chem.* **2002**, *67*, 4945–4950.

(13) Falchi, A.; Giacomelli, G.; Porcheddu, A.; Taddei, M. *Synlett* **2000**, *2*, 275–277.

(14) (a) Han, S.; Moore, R. A.; Viola, R. E. *Bioorg. Chem.* **2002**, *30*, 81–94; (b) Sarabia, F.; Sánchez-Ruiz, A.; Chammaa, S. *Bioorganic Med. Chem.* **2005**, *13*, 1691–1705.

(15) For a more detailed comparison of the ^{1}H and ^{13}C spectra of natural and synthetic aeruginosin KT608A, see Supporting Information page S24.

(16) Valls, N.; López-Canet, M.; Vallribera, M.; Bonjoch, J. *Chem. Eur. J.* **2001**, *7*, 3446–3460.

(17) (a) Wipf, P.; Methot, J. L. *Org. Lett.* **2000**, *2*, 4213–4216; (b) Pierce, J. G.; Kasi, D.; Fushimi, M.; Cuzzupe, A.; Wipf, P. *J. Org. Chem.* **2008**, *73*, 7807–7810.

(18) For the proposed revised structures of aeruginosins KT608B, KT650, GH553, DA495A, DA511 and KB676, see Supporting Information pages S25-S26.